RESEARCHREPORT

SWINE HEALTH

Title: Development and implementation of an on-farm cleaning and disinfection audit
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I. Abstract:

Our goal was to determine whether rapid Lightning™ and BioClean tests used in the food industry to rate sanitation levels could be used in pork production units to evaluate sanitation. Additionally, sanitation audits were performed on a nursery, wean to finish unit, and farrowing house. Swab samples were collected from feeders, flooring, and walls in a wean-finish unit and a nursery room of a 1600 sow farrow-to-finish commercial operation. In both facilities, three 6.16 cm^2 swab samples were collected at each sampling site and analyzed using Lightning™, BioClean, and cultural examination for bacteria. The sensitivity and specificity of Lightning™ and BioClean tests were calculated for each surface using cultural examination as the gold standard for classifying a sample as clean or contaminated. Factors such as feed, manure, and disinfectant residues were tested to determine if they interfered with the sensitivity and specificity of Lightning™ or BioClean tests. Lightning™ tests were generally highly sensitive but lacked specificity. Lightning™ tests falsely classified many clean surfaces as dirty. BioClean tests were generally highly specific but lacked adequate sensitivity. BioClean tests falsely classified many dirty surfaces as clean.

II. Introduction:

All-in, all-out management of confinement units is strongly recommended to maintain herd health and increase profitability. An essential step for implementation of all-in, all-out pig flow is the cleaning and disinfection of rooms between pig groups to prevent exposure to pathogens shed by the previous group.[1,2,3,4] Recommendations include that the room and equipment therein be pressure washed with hot water and a detergent to remove organic matter, and then disinfected with a suitable product.[5,6] Reports have shown that label claims do not always reflect disinfectant efficacy.[7] Consequently, in most cases, an outbreak of neonatal scours or other costly disease must occur before producers and veterinarians will reevaluate cleaning methods and disinfectants used. Currently, producers do not have an objective, rapid, proactive method to

evaluate the final cleanliness of the room, or the efficacy of the disinfectant in inactivating pathogens enzootic to the farm.

Rapid tests to evaluate sanitation levels have not been developed specifically for use on farms. However two testing systems, Lightning™ (BioControl Systems, Inc.; Bellevue, WA) and BioClean (BioVet, St. Anthony, MN) were developed for monitoring food safety in processing plants.[8,9] The Lightning system has recently been used for assessing cleanliness of swine transport vehicles.[10,11] Lightning™ test displays a Lightning™ score 11 seconds after the surface sample is obtained. Each Lightning™ test costs approximately $2.78 after the initial capital outlay for the luminometer. The BioClean test can be prepared in approximately one minute and results are obtained within twenty minutes after sampling. Each BioClean test costs approximately $2.16. To date, neither Lightning™ nor BioClean has been validated for use in pork production facilities.

III. Objectives

1. To determine the most accurate and cost-effective measuring tool for use in the cleaning audit by testing and comparing three tools (Standard bacterial plate counts, Lightning ™, Bio-Clean.)
2. To develop statistically sound procedures for an on-farm cleaning audit to assess room sanitation and disinfectant efficacy on a variety of surfaces.

IV. Procedures:

Objective 1; Experiment 1: Determination of the sensitivity and specificity of Lightning™ and BioClean tests

Study Design:
Swab samples were collected from feeders, flooring, and walls in a wean-finish unit and a nursery room of a 1600 sow farrow-to-finish commercial operation. A wean-finish room was emptied of pigs, cleaned, disinfected (Triphenol-R/256; I.D. Russell Company Laboratories; Longmont, CO, USA), and allowed to dry for one day. Each of 26 pens in the facility was sampled on three surfaces: stainless steel feeder (Farmweld, Teutopolis, IL), concrete slat, and concrete wall. Pigs were also emptied from a 16 pen nursery room. The room was cleaned and disinfected with Triphenol-R/256 and allowed to dry for two days. Samples were taken in each pen from the stainless steel feeder (Farmweld, Teutopolis, IL), plastic mesh flooring (MIK flooring; MIK Heinrich Michel, Marienhausen, Germany), and PVC plastic-covered wall (AP Livestock, Assumption, IL). A sterile metal washer with an internal surface area of 6.16 cm^2 (0.955 in^2) was used to standardize the sampled surface area. In both facilities, three swab samples of a 6.16 cm^2 (0.955 in^2) area were collected at each sampling site and analyzed using Lightning™, BioClean, and cultural examination for bacteria.

Background contamination control samples
Sterile washers were exposed to facility airspace for approximately 5 seconds at 5 geographical locations in each room to quantitate background aerosol contamination of washers that could occur during sampling. The inner surface of each washer was swabbed and analyzed using Lightning™, BioClean, and cultural examination for bacteria as described below. Additionally, metal washers autoclaved in the same batch as those used for on-farm sampling were culturally examined under aseptic conditions to ensure sterility.

Bacterial plating analysis
Swab samples were diluted in 1 mL aliquots of 0.9% saline and placed on ice packs. Original samples and serial dilutions were plated onto trypticase soy agar with 5% sheep blood (BBL® Stacker® Plate; Beckton Dickenson Microbiology Systems, Cockeysville, MD) within 5 hours of collection and incubated at 37°C for 18 hours. Colonies were counted on each plate and converted to colony forming units (CFUs) per cm^2 of surface sampled. The average bacterial count for the 5 background contamination control samples for the room was subtracted from each sample swab count. A surface was classified as either clean ($\leq$1 viable bacterium/cm^2) or contaminated (> 1 viable bacterium/cm^2).[1] Plates indicating more than 487 CFUs/cm^2 surface area were considered Too Numerous To Count (TNTC) and classified as contaminated. Samples that detected less than the mean background contamination level were classified as sterile (0 CFUs/cm^2).

Lightning analysis
The Lightning™ system was used according to test instructions. Briefly, swab samples were bathed with the buffer contained in the Lightning™ swab and then mixed with the luciferin/luciferase pellet to activate the reaction. Residual ATP present on surfaces reacted with the luciferin/luciferase pellet to yield light. The Lightning™ luminometer measured the light output and calculated a score. Each sample was classified by the standard Lightning™ cut-off as either clean (score $\leq$ 2.5) or contaminated (score > 2.5). Alternative cut-off values ($\leq$3.0, $\leq$3.5, $\leq$4.0) were analyzed to determine their effect on test sensitivity and specificity.

BioClean analysis
Sample tubes were prepared according to test instructions by adding 1 drop of reagent B to the tube containing reagent A. Each surface was sampled with a sterile cotton swab provided with the BioClean kit. Protein contaminants reacted with copper ions to form a complex with the biuret reagent that caused a color changing reaction . The sample swab was allowed to react for 20 minutes in the tube containing the reagent mixture. The surface was classified as clean (no color change after 20 minutes) or contaminated (any color change after 20 minutes).

Objective 1; Experiment 2: Factors on pork production units that may interfere with the sensitivity and specificity of Lightning™ or BioClean tests

Study design:

<u>Effect of disinfectant residue on test results</u>

Disinfectant sampling was performed under controlled laboratory conditions to determine if disinfectant residue affected Lightning™ or BioClean test results. Representatives of six classes of disinfectants were used (Table 1). Clean, glass slides (Esco 3"x 1" microscope slides; Erie Scientific Company; Portsmouth, NH) were submerged in disinfectant solution prepared according to label instructions. Slides were dried in a sterile, hepafiltered biosafety cabinet. Dry slides were sampled with each of the three testing methods. The top one-third of the slide was culturally examined for bacteria, the middle one-third for the Lightning™ test, and the bottom one-third for the BioClean test. Five replicates were performed for each treatment. Five control slides were submerged in sterile 0.22µm filtered water and tested as above.

<u>Effect of manure residue on test results</u>

Five manure samples were collected from a growing-finishing facility inhabited by approximately 45 kg (100 lb) pigs and diluted with sterile 0.22µm filtered water to a final dilution of 0.001g manure/mL water. Each sample was divided into two containers. One container was autoclaved for 20 minutes at 250°F (121°C) and the other remained non-sterile. Both the non-sterile manure samples and the autoclaved manure samples were analyzed using cultural examination for bacteria, Lightning™, and BioClean methods.

Effects of feed residue on test results

Five growing-finishing feed samples were collected. Feed samples were diluted to a final concentration of 0.083g feed /mL of 0.22µm filtered water. Each sample was divided into two containers. One container was autoclaved for 20 minutes at 250°F (121°C) and the other remained non-sterile. Both the non-sterile feed samples and the autoclaved feed samples were analyzed using cultural examination for bacteria, Lightning™, and BioClean tests.

Effects of disinfected manure and feed on test results

Five manure samples were collected from a continuous flow finishing facility housing pigs weighing approximately 68 kg (150 lbs). Manure samples were diluted in sterile 0.22µm filtered water to a concentration of 0.01g manure/mL water. Five feed samples of an SEW diet containing carbadox were collected and diluted in sterile 0.22µm filtered water to a concentration of 0.01g feed/mL water. To simulate contamination with organic matter and bacteria, seven slides were submerged in the slurry of manure and seven slides were submerged in a slurry of feed and dried in a sterile, hepafiltered biosafety cabinet. Inoculated slides were then submerged in Clorox® Bleach, 1-Stroke Environ®, Roccal®D-Plus, Cidex® Activated Dialdehyde solution, or Nolvasan® solution solution for 5 seconds and dried a second time. Control slides were submerged in the slurry of manure and feed, respectively and sampled while still wet and again after drying in a sterile hepafiltered biosafety cabinet. All slides were evaluated using cultural examination for bacteria, Lightning™, and BioClean tests.

Data analysis:
The sensitivity and specificity of Lightning™ and BioClean tests were calculated for each surface using cultural examination as the gold standard for classifying a sample as clean or contaminated. Sensitivity was defined as the ability of the test to detect a contaminated surface when the surface was truly contaminated. Specificity was defined as the ability of the test to detect a clean surface when the surface was truly clean.

Objective 2; Experiment 1:

Swab samples were collected from feeders, flooring, and walls in a wean-finish unit and a nursery room of a 1600 sow farrow-to-finish commercial operation, and from a farrowing house in a 235 sow unit.

Sample size: Areas used above will be sampled according to the following chart:

Number of crates/pens per room	**Number of crates/pens to sample**
1-10	Test all
11-35	Test 10
36 or more	Test 30% or 30 whatever number is less

This sample size will enable us to detect at least one contaminated pen in room with 95% confidence if the prevalence of contamination is 10%. Sample sites will be randomly allocated among pens.

Scoring: The percentage of samples designated "clean" for each sampling site (floor, wall, feeder) will be determined. Each sampling site will be scored out of a possible 100 points. The percentages will be averaged to determine the total cleanliness score (out of 100 possible points) for the room. Scores of greater than 90% will be considered good. Scores between 80-89% will be considered satisfactory. Scores of less than 80% will be considered unacceptable. If scores in all areas are unacceptable, then management procedures or disinfectant efficacy must be examined. If a particular area scores poorly (i.e. feeder scores 30%, but walls and floors score 95%), then cleaning procedures for that area (feeders in this example) need to be examined.

V. Results:

An ideal test for assessing sanitation on pork production facilities would be inexpensive, rapid, 100% sensitive, and 100% specific. Lightning™ and BioClean testing systems were not designed for use in pork production facilities. Lightning™ and BioClean technology were transferred to swine production because of the need by the industry to quickly and objectively evaluate sanitation protocols.

Under the conditions of this study, Lightning™ tests were both highly sensitive and specific on PVC plastic-coated walls. However, Lightning™ tests were highly sensitive but lacked specificity when used on other surfaces. One explanation for result variation is that the feeder trough and plastic floor are horizontal surfaces where organic material and bacteria are more likely to remain following rinsing; whereas, material is more easily removed by draining water from a vertical wall. To compensate for increased contamination of swine facilities, some have recommended elevating the Lightning™ cut-off score for classifying a surface as clean. In our study, incremental increases in cut-off scores improved specificity, but decreased sensitivity of Lightning™ for all surfaces except PVC plastic-covered walls. Therefore, altering cut-off scores did not improve overall test accuracy.

Further evaluation of Lightning™ demonstrated that sterile organic material such as autoclaved or disinfected feed and manure residues were sometimes classified as contaminated according to the Lightning™ tests. Feed is made primarily of plant products. Living cells present in the ground seed coats may contain sufficient ATP to cause false positive Lightning™ results. Sterile

manure residue could cause false positive results because of ATP in fibrous seed coats, which pass through the digestive tract of the pig with little degradation. An additional source of ATP in manure could be epithelial cells sloughed from the digestive tract. Pure disinfectant residues did not affect Lightning™ results.

BioClean tests were recently made available for commercial use and have not been widely implemented in the pork industry. Under the conditions of this study, BioClean tests were generally highly specific for all surfaces except plastic flooring, but lacked adequate sensitivity. Neither sensitivity nor specificity was adequate for plastic flooring. Poor overall BioClean sensitivity could result from inadequate reagents in the kit to detect small quantities of protein. An alternative explanation is that residual protein is not a good indicator of bacterial contamination. A second disadvantage of the BioClean test is cross-reaction with Cidex® Activated Dialdehyde solution, an aldehyde disinfectant resulting in the classification of sterile solutions of pure disinfectant as contaminated. However, false classification did not occur when Cidex® Activated Dialdehyde solution was used to disinfect feed or manure samples. One explanation is that the organic material bound the residue in Cidex® that cause false positive reactions. Other classes of disinfectants tested did not affect BioClean test results.

In conclusion, the low specificity of Lightning™ and the low sensitivity of BioClean tests resulted in an inability to accurately assess contamination in swine production facilities. Thus, Lightning™ and BioClean testing systems are not recommended for use in evaluating sanitation levels on swine farms. Future research should focus on validating additional rapid testing systems used by other industries to determine whether they are applicable to the pork industry. Development of rapid testing systems designed for use in the pork industry should be considered if technology transfer from other industries is not possible. Until rapid testing systems can be validated for the pork industry, cultural examination for bacteria remains the gold standard for sanitation audits.

Experiment 2:Sanitation audits:

A. Farrowing house:

Overall Score: Unacceptable -All crates had at least 1 sample classified as dirty.

Floors: (50%) 5 of 10 floors were classified as clean.

Feeders: (40%) 4 of 10 feeders were classified as clean.

Pig mats: (50%) 5 of 10 pig mats were classified as clean.

B. Nursery:

Overall Score: Unacceptable -All pens had at least 1 sample classified as dirty.

Floors: (12.5%) 2 of 16 plastic floors were classified as clean.

Feeders: (25%) 4 of 16 feeders were classified as clean.

Walls: (81%) 13 of 16 pen walls were classified as clean (satisfactory).

C. Wean-to-Finish:

Overall Score: Unacceptable -All pens had at least 1 sample classified as dirty.

Slats: (69%) 18 of 26 floors were classified as clean.

Feeders: (77%) 20 of 26 feeders were classified as clean.

Walls: (69%) 18 of 26 walls were classified as clean.

In conclusion, rooms and equipment that appeared visually clean were not being adequately cleaned and disinfected. Sanitation audits can be used to determine the most effective cleaning procedures and disinfectant for the farm.

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